UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
09/058,323	04/09/98	HOUWEN B	10690/101683

HM22/0703

BRYAN CAVE
245 PARK AVENUE
NEW YORK NY 10167-0034

EXAMINER	
GABEL, G	
ART UNIT	**PAPER NUMBER**
1641	10

DATE MAILED: 07/03/00

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

Office Action Summary

Application No.	Applicant(s)
09/058,323	Houwen et al.
Examiner	**Group Art Unit**
Gailene R. Gabel	1641

☒ Responsive to communication(s) filed on *Apr 10, 2000*

☐ This action is **FINAL.**

☐ Since this application is in condition for allowance except for formal matters, **prosecution as to the merits is closed** in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11; 453 O.G. 213.

A shortened statutory period for response to this action is set to expire *3* month(s), or thirty days, whichever is longer, from the mailing date of this communication. Failure to respond within the period for response will cause the application to become abandoned. (35 U.S.C. § 133). Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

Disposition of Claim

☒ Claim(s) *1-12* is/are pending in the applicat

Of the above, claim(s) _____ is/are withdrawn from consideration

☐ Claim(s) _____ is/are allowed.

☒ Claim(s) *1-12* is/are rejected.

☐ Claim(s) _____ is/are objected to.

☐ Claims _____ are subject to restriction or election requirement.

Application Papers

☐ See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.

☐ The drawing(s) filed on _____ is/are objected to by the Examiner.

☐ The proposed drawing correction, filed on _____ is ☐ approved ☐ disapproved.

☐ The specification is objected to by the Examiner.

☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

☐ All ☐ Some* ☒ None of the CERTIFIED copies of the priority documents have been

☐ received.

☐ received in Application No. (Series Code/Serial Number) _____ .

☐ received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

*Certified copies not received: _____

☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

☐ Notice of References Cited, PTO-892

☐ Information Disclosure Statement(s), PTO-1449, Paper No(s). _____

☐ Interview Summary, PTO-413

☐ Notice of Draftsperson's Patent Drawing Review, PTO-948

☐ Notice of Informal Patent Application, PTO-152

— SEE OFFICE ACTION ON THE FOLLOWING PAGES —

DETAILED ACTION

Amendment Entry

1. Applicants' amendment and response filed 4/10/00 in Paper No. 9 is acknowledged and has been entered. Claims 1-12 have been amended. Currently, claims 1-12 are pending and under examination.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

2. Claims 1-12, as amended, are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 1-12 are vague and indefinite in reciting language inconsistent with accepted meaning of the terms. In this case, the terms "staining" and "labeling" are used interchangeably by the Applicants, rendering the claims unclear.

While applicant may be his or her own lexicographer, a term in a claim may not be given a meaning repugnant to the usual meaning of that term. See *In re Hill*, 161 F.2d 367, 73 USPQ 482 (CCPA 1947). The accepted meaning of "staining" is to color, to dye, or to stain

directly a biological element using standard histologic techniques, i.e. propidium iodide directly introduced into the nucleus of an erythroblast for staining, thus "stained erythroblast". Alternatively, the accepted meaning of "labeling" is the incorporation of a substance that is detectable such as a label (FITC or radionuclide) into a biological material using standard immunological techniques such as coupling a fluorescent label to an antibody to specifically bind an antigen, thus "fluorescently-labeled leucocytes" via mediation of a specific antibody.

Accordingly, claim 1 is indefinite in reciting "staining leucocytes".

Claim 1 (i) is indefinite in reciting "by (plainly) adding a fluorescent labeled leucocyte binding antibody to the hematologic sample" because it fails to specifically define the functional interactive relationship between the leucocytes and the fluorescent labeled leucocyte binding antibody. For example, language such as "staining ... by adding ... to the hematologic sample to bind the leucocytes" is suggested but not required to obviate indefiniteness in the claim.

Claim 1 (iv) is vague and indefinite in reciting "the nucleotide fluorescent signal of the erythroblasts and the fluorescent labeled antibody signal of the leucocyte" it does not specifically and clearly define the structural and functional cooperative relationship between the elements, i.e. signals, stains/labels, and cells. For example, language such as "the nucleotide fluorescent signal of the stained erythroblasts and the fluorescent signal of the labeled antibodies bound to the leucocytes" is suggested but not required in clarifying the claim.

Claim 3, lines 1-3 is confusing in reciting "wherein labeled leucocyte binding the fluorescent of the fluorescent labeled antibody in the step (I) comprises".

In claim 3, change "CY5 stands for a arylsulfonate cyanine fluorescent dye" to --arylsulfonate cyanine fluorescent dye or CY5-- to properly define CY5.

Claim 4 (ii) is indefinite and confusing in reciting "a pH wherein the leucocytes are stained" because it is unclear how the pH (acidic vs neutral) effects the "labeling" of the leucocytes especially that the labeling of the leucocytes has already been effected in step (i) of claim 1. Further, the permeability modulation is specifically directed to the erythroblasts, the acidic pH range is controlled to prevent lysis of all cells including the labeled antibody bound to the leucocytes, and the compensating agent is added to retain the shape and integrity of the leucocytes. For example, does the pH affect the binding of the fluorescent labeled antibody to the surface antigen of the leucocytes? Please clarify. Alternatively, claim 4 lacks a critical limitation in not clearly defining what pH is more conducive or suitable for the staining of nuclei of the erythroblasts in step (iii) of claim 1. See pages 10-14 of the specification.

Claim 4 (ii) has improper antecedent basis problem in reciting "adjusting a mixture of the hematologic sample and the first reagent fluid". Change to --adjusting the mixture of the hematologic sample and the first reagent fluid-- for proper antecedent basis.

In claim 10, insert --method-- between "The" and "according to claim 5,"

Claim 10 is vague and indefinite in reciting "to stain erythroblasts according to degrees of maturity of the erythroblasts... erythroblasts are classified into at least two groups according to the degrees of maturity" in relation to the concentration of the nucleotide fluorescent dye. Do the Applicants intend to mean increasing or decreasing the concentration of the dye (within the given

range) depending on the maturity of the erythroblasts. Accordingly, the relationship between the dye concentration and the level of maturity of the erythroblasts is unclear.

In claim 12, line 9, remove --of-- between "The method" and "according to claim 11."

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103© and potential 35 U.S.C. 102(f) or (g) prior art under 35 U.S.C. 103(a).

3. In light of applicants' amendment and arguments, the rejection to claims 1-3 and 5- 9 under 35 U.S.C. 103(a) as being unpatentable over Kim et al. (US 5,559,037) in view of Loken et al. (US 5,047,321) is, hereby, withdrawn.

4. In light of applicants' amendment and arguments, the rejection to claims 1-12 under 35 U.S.C. 103(a) as being unpatentable over Inami et al. (US 5,298,426) in view of Loken et al. (US 5,047,321)is, hereby, withdrawn.

New Grounds of Rejection

5. Claims 1-12 are rejected under 35 U.S.C. 103(a) as being unpatentable over Loken et al. (US 5,047,321) in view of Kim et al. (US 5,559,037) and Inami et al. (US 5,298,426).

Loken et al. has been discussed in Paper No. 4. Specifically, Loken et al. disclose a method comprising combining a body fluid sample such as whole blood with at least two nucleotide fluorescent dyes such as RNA dye or DNA dye and at least one fluorescent labeled antibody or cell surface marker to form a labeled mixture. The dyes independently and differentially assess different characteristics of nucleated cells in the sample and the fluorescent labeled antibody is specific for a cell surface antigen differentially expressed on cells of different lineages, i.e. leucocytes (see column 4, lines 27-40). Each of the dye and label is fluorescent, excitable at the same wavelength and has a peak emission spectra that is distinguishable from the others. Loken et al. exemplifies use of phycoerythrin (PE) fluorescent label (see column 5, lines 16-20). The labeled hematologic mixture is measured and analyzed using flow cytometric measurements of fluorescence intensity and light scatter for each cell examined.

Loken et al. fail to disclose increasing permeability of cytoplasm of specific nucleated cells, specifically erythroblasts using a materials such as those in claim 4 of the instant invention prior to incorporating RNA or DNA dyes thereto.

Kim et al. has been discussed in Paper No. 4. Specifically, Kim et al. disclose simultaneous and quantitative, flow cytometric analysis of erythroblasts and leucocytes. Kim et al. teach mixing an aliquot of the blood sample with diluent which rapidly destroys the cytoplasm (lyses) of erythroblasts and erythrocytes and allowing exposure of erythroblastic nuclei while preserving the integrity and shape of the cytoplasm of leucocytes (see column 4, lines 60-65). Kim et al. further disclose exposing nucleotide fluorescent dye such as YOYO-1, YOYO-3, TOTO-1, TOTO-3, BO-PRO-1, YO-PRO-1, TO-PRO-1, Propidium iodide, ethidium bromide to erythroblasts while minimizing its permeation into leucocytes (see column 2, lines 40-48 and column 6, lines 36-57). Kim et al. constructs a three-dimensional plot of qualified intensity signals of fluorescence and scattered light from detected signals to differentiate and quantitate erythroblasts and leucocytes after flow cytometric analysis.

Inami et al. has been discussed in Paper No. 4. Specifically, Inami et al. disclose a two-step method of differentiating erythroblasts from leucocytes. Inami et al. specifically disclose mixing blood with a hypotonic fluorescent dye solution capable of diffusing into erythroblasts to stain their nuclei and a buffer for maintaining the pH in the acidic range. Inami et al. further disclose mixing the (acidic) sample mixture with a second fluid comprising a buffer that neutralizes the acidic pH in the solution to a staining pH and an osmolarity adjusting agent for

adjusting the osmolarity of the solution to a value at which the shape and integrity of leucocytes are maintained (see column 2, lines 3-24 and column 4, lines 17-41). The first acidic and hypotonic fluid has a low osmolality causing erythrocytic cell lines in the sample to swell upon absorbing water causing cellular contents to leak out and nucleotide fluorescent dye to diffuse through the cell membrane to stain the nuclei. Leucocytes do not permit the entrance of nucleotide fluorescent dye (see column 5, line 60 bridging to column 6, line 26). Inami et al. enumerates the different dyes used in the first fluid for differentiating leucocytes and erythroblasts, including propidium iodide and ethidium bromide specific for erythroblast nuclei, and appropriate concentrations thereof in column 3 of the disclosure. Inami et al. disclose that a the concentration of nucleotide fluorescent dye, i.e. propidium iodide or ethidium bromide, should fall within the range of 0.003 mg/L to 10 mg/L (2.5 μg/ml to 100μg/ ml) in order to achieve optimum results (see column 4, lines 5-16). Inami et al. also suggest incorporating leucocyte staining dyes in the first fluid (see column 6, lines 13-18). After treatment, stained cells are measured using a flow cytometer and erythroblasts are separated from other cell groups on the resulting two-dimensional plot where erythroblasts are counted (see column 6, lines 9-12). Figure 9 shows a two-dimensional plot showing selective staining of erythroblasts with nucleotide staining dye to emit red fluorescence and to permit erythroblasts to be distributed in a separate zone from other cells so that the relative content and count can be determined. Figure 10 and 11 show two-dimensional plots for the intensity of red fluorescence versus the intensity of side-scattered light obtained for peripheral blood and bone marrow.

It would have been obvious to one of ordinary skill in the art at the time of the invention to combine teachings of Kim in differentially identifying erythroblasts using nucleotide fluorescent dyes with the method of Loken in differentiating between nucleated hematopoietic cells and subclasses using nucleic acid dyes and fluorescent labeled antigen specific antibodies and incorporate therewith, the method of Inami wherein blood samples are treated to effect selective permeability of erythroblast cytoplasm to nuclear dyes because Loken specifically expressed the need to analyze, discriminate, and count various populations of cell types which is not merely limited to leucocytes and Kim, likewise, expressed such a need including specifically counting and differentiating erythroblast populations in blood and bone marrow and Inami specifically suggested incorporating different dye elements into his method in order to allow differentiation of both erythroblasts and leucocytes. One of ordinary skill in the art at the time of the instant invention would have been motivated to combine the teachings of Kim and Inami with the method of Loken in differentiating nucleated hematopoietic cells and subclasses using nucleic acid dyes and fluorescent labeled antigen specific antibodies because it allows for simultaneous and accurate differentiation and counting of both erythroblasts and leucocytes in a whole blood or bone marrow sample. Another motivation includes affording reduced analytical time and accuracy in discriminating and counting of erythroblasts and leucocytes which are specific diagnostic indicators of diseases such as anemia and leukemia.

Remarks

6. Applicant's arguments with respect to claims 1-12 have been considered but are moot in view of the new ground of rejection.

Alternatively, in addressing applicants' remarks on each separate individual prior art reference used, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986).

Further, in addressing applicants' remarks with regards to combining the above prior art references, note the aforementioned new ground of rejection. It must be recognized that any judgment on obviousness is in a sense necessarily a reconstruction based upon hindsight reasoning. But so long as it takes into account only knowledge which was within the level of ordinary skill at the time the claimed invention was made, and does not include knowledge gleaned only from the applicant's disclosure, such a reconstruction is proper. See *In re McLaughlin*, 443 F.2d 1392, 170 USPQ 209 (CCPA 1971).

7. Attached, herewith, is a copy of pages from Stedman's Medical Dictionary to assist applicants' in differentiating use of the terms "staining" and "labeling" in drafting/amending the claims.

8. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Gail Gabel whose telephone number is (703) 305-0807. The examiner can normally be reached on Monday to Thursday from 7:00 AM to 4:30 PM. The examiner can also be reached on alternate Fridays from 7:00 AM to 3:30 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Long Le, can be reached on (703) 308-4027. The fax phone number for the organization where this application or proceeding is assigned is (703) 308-4242.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (703) 308-0196.

6/27/00

Gail Gabel
Patent Examiner
Group 1641

LONG V. LE
PRIMARY EXAMINER
A.U. 1641